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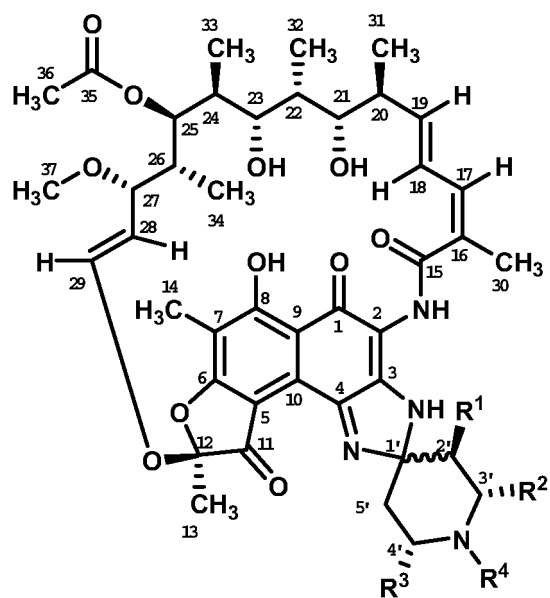
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E-33423 Llanera (ES)(74) Representative: **Barlocchi, Anna****Zea, Barlocchi & Markvardsen, S.L.****Balmes, 114 4****08008 Barcelona (ES)**(54) **SPIROPIPERIDYL RIFAMYCINS FOR THE TREATMENT OF MICROBACTERIAL INFECTIONS**

(57) The compounds of formula (1), their pharmaceutically acceptable salts and their solvates, wherein R¹ is a radical selected between hydrogen and alkyl; R² is selected from hydroxyalkyl, phenyl, phenyl mono-substituted and phenyl di-substituted in positions 3 and 4; R³ is selected from phenyl, phenyl mono-substituted and phenyl di-substituted in positions 3 and 4; the substituents of the phenyl of R² and R³ selected from halogen, (C₁-C₄)-alkyl and (C₁-C₄)-alkoxyl and R⁴ is selected from hydrogen, alkyl, allyl and homoallyl, they are useful for the treatment of mycobacterial infections, in particular for the treatment of infections produced by Mycobacterium tuberculosis, Mycobacterium avium-intracellulare complex, or Mycobacterium kansasii.

EP 1 783 129 A1



(1)

Description

[0001] This invention is related to new rifamycin derivatives from the spiropiperidylrifamycins family, which exhibit a high antibiotic activity, and also a procedure for their preparation.

BACKGROUND ART

[0002] Multiresistant tuberculosis is defined as that tuberculosis which is caused by strains of *Mycobacterium tuberculosis* resistant simultaneously to at least isoniazid and rifampicin. Its incidence has increased in the last number of years in an alarming rate in a numerous regions in the worldwide. The importance of this problem is illustrated in the decision taken by the WHO in 1994 to put into action a plan for the control and surveillance of multiresistant tuberculosis at a worldwide level (cfr. Guidelines for surveillance of drug resistance in tuberculosis WHO/TB/2003.320-WHO/CDS/RMD/2003.3). Among the strategies that have been developed against this serious problem which threatens worldwide health, is the development of new drugs to fight the types of strains involved.

[0003] Drugs for tuberculosis treatment are divided in "first line drugs" and "second line drugs". The first line, is, of greatest effectiveness, and plays an indispensable role in primary therapeutic regimes to guarantee the treatment of this disease. Only four drugs make up the first group, which are: isoniazid, rifampicin, pyrazinamide, and ethambutol. The rest of the drugs nowadays available are included within the category of "second line drugs".

[0004] For the treatment of multiresistant tuberculosis drug combinations are administered over prolonged periods of time. Nowadays, the choice treatment lasts six months and contemplates administration of a combination of isoniazid, rifampicin and pyrazinamide for two months, followed by the administration of isoniazid and rifampicin combination for four subsequent months. It is of vital importance to develop new drugs which permit reinforcement or improvement in the treatment of multiresistant tuberculosis.

[0005] On the other hand, related to the HIV epidemic and due to the generalized use in modern medicine of antitumour and immunosuppressor drugs, there have been increased infections produced by the named "atypical mycobacteria" (also known as "opportunistic environmental mycobacteria" or as "nontuberculous mycobacteria"). This term includes numerous different species from whose method of transmission is not known, nor is their resistance mechanisms to antimycobacterials. These atypical mycobacterias behave as microorganisms usually resistant to the drugs with antimycobacterial activity and pose a serious threat. From the numerous species isolated, *Mycobacterium avium-intracellulare* complex stands out for its frequency of producing serious disseminated infections and producing a pulmonary pathology undistinguishable from the one produced by *M. tuberculosis*. *Mycobacterium avium-intracellulare* complex behaves as multiresistant to the drugs of the "first" and "second line". In the therapeutic regimes the rifabutin is generally the elective treatment, associated to a macrolide and to a quinolone. The high toxicity in vivo of the rifabutin represents a common inconvenience. *Mycobacterium kansasii* usually has a pattern of sensitivity much more variable, even though it exhibits resistance to isoniazid and ethambutol.

[0006] Rifamycins are natural chemical substances that were isolated for first time in 1959 from cultures of *Nocardia Mediterranei* as a complex mix of rifamycins A-E (cfr. P. Sensi et al., *Il Fármaco*, Ed. Sc. 1959, vol. 14, pp. 146-147). They belong to the ansamycin family, antibiotics of great interest against Gram-positive bacteria and mycobacteria such as *M. tuberculosis*. They also have activity against the DNA-dependent bacterial RNA polymerase (cfr. C. Bartolucci et al., *Fármaco* 1992, vol. 47, p. 1367; C. Bartolucci et al., *Pharm. Pharmacol. Lett.*, 1993, vol. 3, p. 1).

[0007] The structure of the rifamycins was determined by chemical degradation of the rifamycin S and by NMR spectroscopics studies. They present as common structural characteristic an aliphatic chain of 17 carbon atoms, called ansa, which connects two non adjacent positions of an aromatic plane chromophore core of naphthohydroquinone linked by an amide group.

[0008] The activity of these compounds is a consequence of the specific inhibition of the bacterial RNA polymerase through the formation of a very stable complex 1:1 between the drug and the enzyme, as it is shown in an investigation of the rifampicin. It has been proposed in a model in which the enzyme wraps the drug by the hydrophilic face and under the aromatic core, in such a way that the positions C₃ and C₄ do not participate in the joining. The studies of structure-activity relationship carried out with different rifamycins conclude that in order to maintain the inhibitory activity the following structural elements are necessary: a hydroxyl group or ketone in C₁ and C₈, hydroxyl groups in position C₂₁ and C₂₃, a determined spatial relation among the functional groups and an ansa bridge (cfr. P. Sensi, *P. Appl. Chem.* 1975, vol. 41, pp. 15-29).

[0009] Hundreds of semisynthetic rifamycins have been prepared with the hope of obtaining substances with better biological activities; most of them present modifications in the positions C₄ (rifamide) and C₃ (rifampicin). These structural changes do not affect the action of the substances over the enzyme in a critical way, but modify other important parameters such as the cellular membrane permeability, the pharmacokinetic properties and the resortion (cfr. S. Lancini et al., "Structure-Activity Relationship among the Semisynthetic Antibiotics". D. Perlmann, Ed., Academic Press, N.Y. 1977, pp. 531-600).

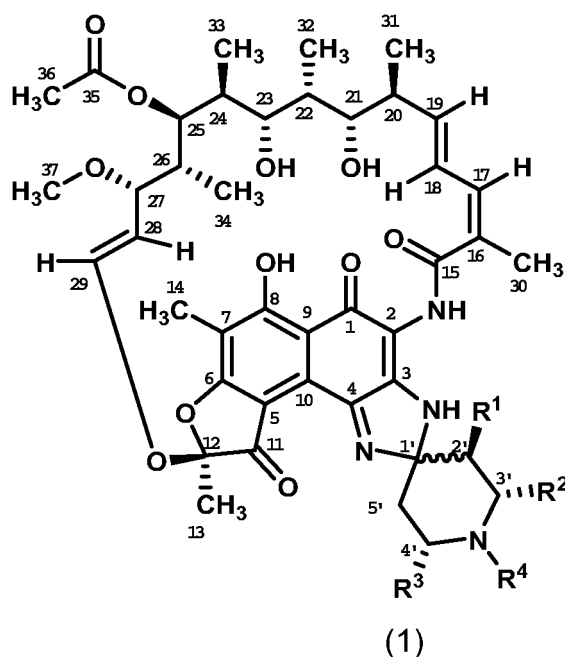
[0010] The spiropiperidylrifamycins are a class of semisynthetic antibiotics derived from the rifamycins in which the carbons C₃ and C₄ are incorporated into an imidazol ring, which itself has a spirocyclic piperidine (cfr. A. Sanfilippo et al., J. Antib. 1980, vol. 33, p. 1193; L. Marsili et al., J. Antib. 1981, vol. 34, p. 1033). The nitrogen atom from the piperidine ring may have different substituents (linear and ramified alkyl radicals, functionalized alkyl, benzyl, acetyl and ethoxycarbonyl) (cfr. US 4.086.225; DE 2.825.445-A). Among these spiropiperidylrifamycins, the rifabutin has been used with therapeutic purposes as a multidrug for the treatment of tuberculosis and against the *Mycobacterium Avium-Mycobacterium intracellulare Complex* (MAC) infections in patients with HIV (cfr. US. 4.219.478).

[0011] From what is known in the art, it is very desirable to provide new drugs possessing biological activity, not only against *Mycobacterium tuberculosis*, but also against mycobacterial infections in general.

SUMMARY OF THE INVENTION

[0012] The inventors have found new structural derivative of spiropiperidylrifamycins with antimycobacterial activity that is comparable and, in many cases better, than the one shown by the existing drugs at present time.

[0013] So, according to an aspect of the present invention it is provided a compound of formula (1), their pharmaceutically acceptable salts and their solvates, including hydrates, wherein: R¹ is a radical selected from hydrogen and (C₁-C₄)-alkyl; R² is selected from the group consisting of hydroxy-(C₁-C₄)-alkyl, phenyl, phenyl mono-substituted phenyl, and phenyl di-substituted in positions 3 and 4, being the phenyl substituents selected from the group consisting of halogen, (C₁-C₄)-alkyl and (C₁-C₄)-alkoxyl; R³ is selected from the group consisting of phenyl, phenyl mono-substituted phenyl, and phenyl di-substituted in positions 3 and 4 being the phenyl substituents selected from the group consisting of halogen, (C₁-C₄)-alkyl and (C₁-C₄)-alkoxyl; and R⁴ is selected from the group consisting of hydrogen, (C₁-C₄)-alkyl, allyl and homoallyl.



[0014] In a preferred embodiment, the compounds of formula (1) are those wherein, when R² and R³ are phenyl mono- or di-substituted, the substituents of the phenyl are independently selected from halogen and (C₁-C₄)-alkoxyl. In a much preferred embodiment, the substituent is the methoxyl.

[0015] The compounds of formula (1) especially preferred of the present invention are shown in Table 1. Said compounds have been denominated in the present specification indistinctly as compounds of formula (1) or as 1 RFA.

Table 1:

Comp. 1	R ¹	R ²	R ³	R ⁴	Tr (h)	Rto. (%)	Rel. Diast. (M:m) crude
RFA 1	Me	CH ₂ OH	Ph	H	48	27	90:10

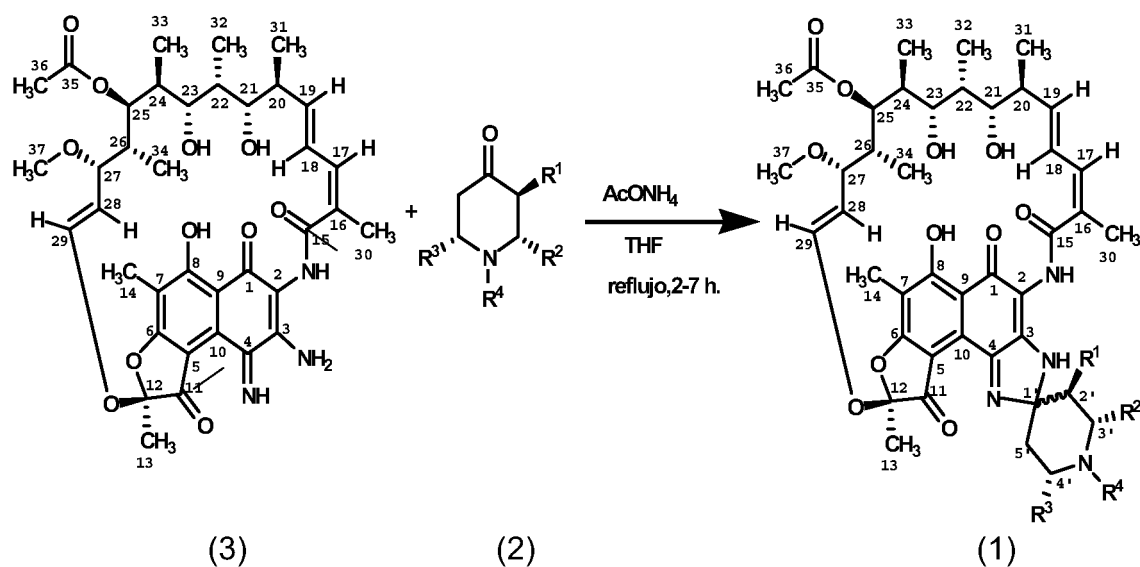
(continued)

Comp. 1	R ¹	R ²	R ³	R ⁴	Tr (h)	Rto. (%)	Rel. Diast. (M:m) crude
RFA 2	Me	CH ₂ OH	4-MeO-Ph	H	48	33	90:10
RFA 3	H	Ph	Ph	H	5	71	68:32
RFA 4	H	4-MeO-Ph	4-MeO-Ph	H	3	69	74:26
RFA 5	H	4-F-Ph	4-F-Ph	H	3.5	70	75:25
RFA 6	H	2-I-Ph	2-I-Ph	H	6	75	80:20
RFA 7	H	Ph	Ph	Allyl	1.5	68	73:27
RFA 8	H	4-MeO-Ph	4-MeO-Ph	Allyl	2	65	78:22
RFA 9	H	3,4-di-MeO-Ph	3,4-di-MeO-Ph	Allyl	3	62	76:24
RFA 10	H	4-F-Ph	4-F-Ph	Allyl	3	69	76:24
RFA 11	H	4-Cl-Ph	4-Cl-Ph	Allyl	4.4	67	74:26
RFA 12	H	2-Br-Ph	2-Br-Ph	Allyl	2	63	75:25
RFA 13	H	3-Br-Ph	3-Br-Ph	Allyl	3	63	80:20
RFA 14	H	2-I-Ph	2-I-Ph	Allyl	5	69	76:24
RFA 15	H	Ph	Ph	homo-allyl	1.5	60	73:27
RFA 16	H	Ph	Ph	Bu	4	62	75:25

[0016] In Table, M means majority diastereoisomer and m means minority diastereoisomer.

[0017] According to another aspect of the present invention, it is provided a process for the preparation of the compound of formula (1) defined previously, which comprises carrying out a condensation reaction between the substituted 4-piperidones of formula (2) and the intermediate 3-amino-4-iminorifamycin S of formula (3). The procedure takes place in soft conditions and with good yield. Schema 1 illustrates one way of accomplishment of said procedure.

Schema 1:



[0018] The intermediate of formula (3) can be obtained from the Rifamycin S (cfr. US 4.017.481 and DE 2,825, 445-

A). The 4-piperidones of formula (2) can be prepared by known procedures (cfr J. Barluenga et al., J. Org. Chem. 1993, vol. 58, pp. 3391; J. Barluenga et al., J. Org. Chem. 1998, vol. 63, p. 3918; A-B. García et al., Tetrah. Letter 2004, vol. 45, p. 4357]. Said 4-piperidones of formula (2) can be disubstituted or trisubstituted. The preferred compounds of formula (2) are shown 2.

Table 2.- Preferred 4-piperidones of formula (2):

Compound 2	R ¹	R ²	R ³	R ⁴
2a	Me	CH ₂ OH	Ph	H
2b	Me	CH ₂ OH	4-MeO-Ph	H
2c	H	Ph	Ph	H
2d	H	4-MeO-Ph	4-MeO-Ph	H
2e	H	4-F-Ph	4-F-Ph	H
2f	H	2-I-Ph	2-I-Ph	H
2g	H	Ph	Ph	Allyl
2h	H	4-MeO-Ph	4-MeO-Ph	Allyl
2i	H	3,4-di-MeO-Ph	3,4-di-MeO-Ph	Allyl
2j	H	4-F-Ph	4-F-Ph	Allyl
2k	H	4-Cl-Ph	4-Cl-Ph	Allyl
2l	H	2-Br-Ph	2-Br-Ph	Allyl
2m	H	3-Br-Ph	3-Br-Ph	Allyl
2n	H	2-I-Ph	2-I-Ph	Allyl
2o	H	Ph	Ph	homoallyl
2p	H	Ph	Ph	Bu

[0019] Preferably, the procedure is carried out in a non-halogenated solvent, and in presence of a base. More preferably, the solvent is a ether, and still more preferable the ether is tetrahydrofuran (THF). Also preferably, the base used is ammonium acetate. The reaction is carried out at a temperature comprised between the room temperature and the reflux temperature of the solvent. More preferably, at the reflux temperature of the solvent. The reaction is monitored until there is no detection of the intermediate 3-amino-4-iminorifamycin S. Said monitoring can be carried out by HPLC (High Performance Liquid Chromatography) and TLC (Thin Layer Chromatography).

[0020] The spiropiperidylrifamycin derivatives obtained are purified from the crude of reaction and isolated through conventional techniques as crystallization or freeze drying.

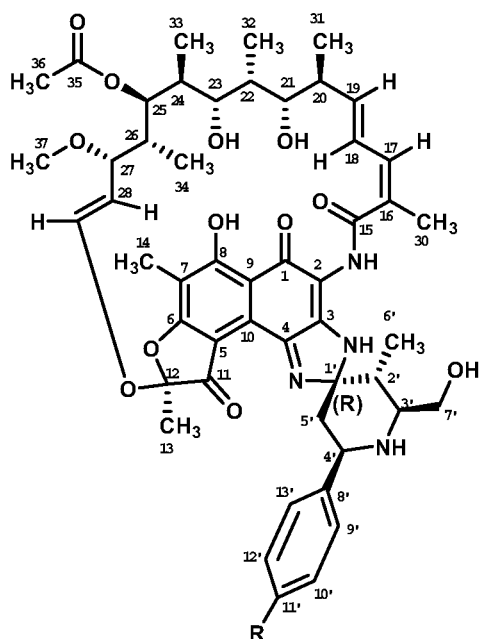
[0021] The basic nature of the compounds of general formula (1) allows their isolation in the form of their pharmaceutically acceptable salts. The compounds of formula (1) can be converted into their salts and the salts can be converted into the free compounds, through conventional methods.

[0022] This procedure of synthesis of spiropiperidylrifamycin derivatives of formula (1) gives rise to the formation of a new stereogenic centre in the spiranic carbon atom C-1'. It constitutes the first known reaction, in which enantiomerically pure 4-piperidones are used in the condensation reaction with the intermediate 3-amino-4-iminorifamycin S, as well as of the use of substituted 4-piperidones in the positions 2, 3 or 6 of the ring.

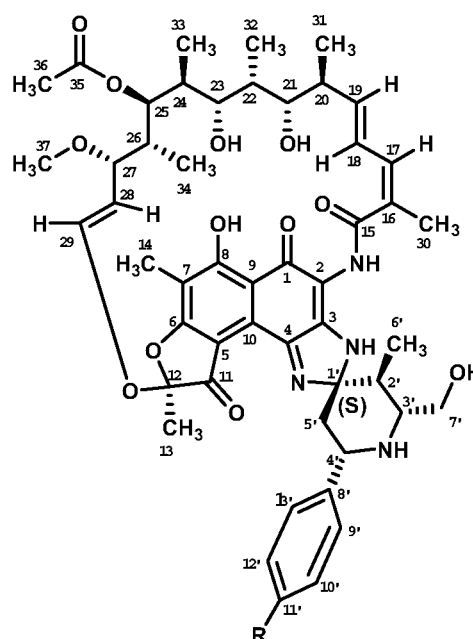
[0023] As an example, the reaction of condensation carried out with the enantiomeric trisubstituted 4-piperidones (-)-(2R,3S,6S)-2-hydroxymethyl-3-methyl-6-phenyl-4-piperidone (2a) and (-)-(2R, 3S,6S)-2-hydroxymethyl-3-methyl-6-p-methoxyphenyl-4-piperidone (2b) provides the corresponding spiropiperidylrifamycins of formula (1) as a mixture of diastereoisomers (majority (M)/ minority (m)) respect to the spiranic carbon C-1'. The proportion of each one is determined by HPLC. In Schema 2 the structure of the diastereoisomers is shown. The mixture of diastereoisomers can be separated and isolated through TLC preparative.

[0024] Exhaustive studies 2D of ¹H and ¹³C-NMR, based on the spectra of gHSQC and gHMBC, have been undertaken to assign all the signals of the molecule, as well as the configuration of the new stereogenic centre, the spiranic carbon C-1', observing a higher screening (minor values of ppm) in the hydrogen signal of the NH-3 in the spectrum of ¹H-NMR of the majority diastereoisomer in relation with the minority (between 0.6 and 1.4 ppm). (Cfr. E. Rubio et al., Maqn. Reson. Chem. 2005, vol.43, pp.269-282).

Schema 2.- Structure of the diastereoisomers



1 RFA 1M (R= H)
1 RFA 2M (R= OCH₃)



1 RFA 1m (R= H)
1 RFA 2m (R= OCH₃)

[0025] According to another aspect of the present invention it is provided a pharmaceutically composition for the treatment of mycobacterial infections, that comprises a therapeutically effective quantity of the compound of formula (1) defined previously, together with suitable quantities of pharmaceutically acceptable excipients or carriers.

[0026] The compounds of formula (1) show a strong biological activity. The Examples 33-34 of this specification show the results obtained in the biological activity study. The assayed compounds show *in vitro* a strong antimycobacterial general activity, even higher than rifampicin-rifabutin in the reference strain *Mycobacterium tuberculosis* ATCC 37837. Several compounds of formula (1) were also assayed against five strains of *Mycobacterium avium-intracellulare* complex, being the five strains sensitive at least to three of the compounds of formula (1) studied. These strains show resistance to rifampicin-rifabutin. With respect to the four strains of *Mycobacterium kansasii* all the compounds of formula (1) studied, had a similar behaviour to rifampicin-rifabutin.

[0027] So, another aspect of the present invention refers to the use of the compound of formula (1) defined previously, for the preparation of a drug for the treatment of a mycobacterial infection, preferably for an infection caused by the following bacteria: *Mycobacterium tuberculosis*, *Mycobacterium avium-intracellulare* complex or *Mycobacterium kansasii*.

[0028] It also belongs to the present invention a method of treatment of a mammal, including a human, that suffers a mycobacterial infection, preferably caused by the following bacteria: *Mycobacterium tuberculosis*, *Mycobacterium avium-intracellulare* complex or *Mycobacterium kansasii*, which comprises the administration to said mammal a therapeutically effective quantity of the compound of formula (1) defined previously, together with suitable quantity of pharmaceutically acceptable excipients or carriers.

[0029] Throughout the description and claims the word "comprise" and its variants is not intended to exclude other technical features, additives, components or steps. The abstract of this application is incorporated herein as reference. For those skilled in the art, other objects, advantages and features of the invention will become apparent deduced in part through the description and in part by practice of the invention. The following examples are provided by way of illustration, and are not intended to be limiting of the present invention.

EXAMPLES

[0030] The nuclear magnetic resonance spectra and the studies of bidimensional correlation were carried out in a Bruker AV-400 operating in a frequency of 400.13 y 100.61 MHz for ^1H and ^{13}C respectively, using a reverse probe QXI $^1\text{H}/^{13}\text{C}/^{15}\text{N}/^{31}\text{P}$ de 5 mm including a bovine of gradient in the axis Z. The samples prepared contained among 30-50 mg of compound. Deuterated benzene (C_6D_6) was used, except in the case of the compound 1 RFA-1m in which deuterated chlorophorm (CDCl_3) was used as solvent. The chemical displacements are expressed in ppm (parts per million) and are referred to a residual signal of the solvent (δ 7.16 for ^1H and of 128.39 for ^{13}C).

[0031] The high resolution mass spectra were carried out in a Finnigan-Matt 95, using electronic impact procedures, electro spray positive (ES pos.) and FAB+. The fusion points have not been corrected and were measured in a Büchi-Tottoli apparatus. The thin layer chromatography (TLC) was done over silica gel plates (Merck 60 G). For the column chromatography silica gel 60 (230-240 mesh) as stationary phase was used. The preparative chromatography was carried out using glass plates (20 x 20 cm) of silica gel (Merck 60 F₂₅₄).

[0032] In the case of the products 1 RFA-1 y 1 RFA-2, the crudes from the reactions were purified by HPLC using a Waters LC Module I plus apparatus, at room temperature, using a detector V-UV Photodiode-Array and a reverse phase column (Kromasil 100 C8 5 μm 25x 1.0). The mobile phase used was a mixture 1:1 of acetonitrile : phosphate buffers, $(\text{NH}_4)\text{H}_2\text{PO}_4$ (0.05 M) at pH 6.8 and flow of 5.5 ml/min.

General method for the preparation of the compounds of formula (1): 1 RFA-1 and 1 RFA-2:

[0033] 100 mg of piperidone (2a-b) are dissolved in 15 ml of tetrahydrofuran (THF) at room temperature, next 2 equivalents of ammonium acetate and one equivalent of the intermediate 3-amino-4-iminorifamycin S (3) are added. The reaction mixture is stirred at reflux for 24 hours. After this time, the reaction mixture is allowed to reach room temperature and an addition is made of 0.5 equivalents more of the intermediate 3-amino-4-iminorifamycin S (3) and the mixture is stirred at reflux for 24 hours more. After this time the mixture is allowed to reach room temperature and diethyl ether (30 ml) is added, it is filtered over celita and it is washed again with diethyl ether (2 x 15 ml). The organic phase is washed with a buffer solution (pH 7,6), of $(\text{NH}_4)\text{H}_2\text{PO}_4$ 0.05 M (3 x 15 ml), it is dried with sodium sulphate anhydrous and concentrated to dryness. The solid obtained is a mixture comprised mainly of the excess of the intermediate 3-amino-4-iminorifamycin S (3) and the new compounds of formula (1), which are washed with acetic acid at 4% (as many times as necessary so that TLC does not appear violet) and passed to the aqueous phase in acetate form while the precursor is kept in the etheria phase. The violet aqueous phase is extracted with chloroform, leaving long decantation periods. The organic layer therefore obtained, it is washed with the buffer solution mentioned previously (portions of 30 ml) until the waters of washing have a neutral pH. The organic phase is dried with sodium sulphate anhydrous and filtered. The solvents are evaporated at reduced pressure and it is obtained a residue that corresponds mainly to the mixture of diastereoisomers (90:10), which are separated by HPLC using as eluent a mixture phosphates buffer: acetonitrile 1:1 at pH 6.8. The products are isolated by chloroform extraction (CHCl_3), dried over sodium sulphate anhydrous, filtered and evaporation of the selected chromatographic fractions. The products are presented as violet solids. The product is isolated by freeze drying, extraction and evaporation or crystallization of the selected chromatographic fractions

[0034] Following this general method the compounds of the Examples 1 to 4 were prepared.

Example 1: Preparation of 4-deoxo-3,4-[(2R)-2-spiro(6-phenyl-2-hydroxymethyl-3-methyl-4-piperidil)-2,5-dihydro-1 H-imidazo]rifamycin S (1 RFA-1M).

[0035] Following the general procedure of RFA (1-2) from the compound 2a shown in Table 2 and the compound (3) shown in Schema 1, the compound of the title was obtained that presents the following data: HRMS (ESI pos): 911.4425 [calculated for $(\text{M}+1)^+$: 911. 4437]. Elemental anal.: calculated (%) for $\text{C}_{50}\text{H}_{62}\text{N}_4\text{O}_{12}$: C 65.92; H 6.86; N 6.15; found: C 65.81; H 6.92; N 6.19. HPLC: t = 12,1 min (ϕ = 1 ml/min; phosphates buffer: acetonitrile 1:1 a pH = 6.8). TLC:Rf: 0,20 (toluene:methanol: ethyl acetate 6:1:1). ^1H -RMN: 9.10 (NH-2); 8.85 (NH-3).

Example 2: Preparation of 4-deoxo-3,4-[(2S)-2-spiro(6-phenyl-2-hydroxymethyl-3-methyl-4-piperidil)-2,5-dihydro-1 H-imidazo]rifamycin S (1 RFA-1m).

[0036] Following the general procedure of RFA (1-2) from the compound 2a shown in Table 2 and the compound (3) shown in Schema 1, the compound of the title was obtained that presents the following data:P.f.:159-62 °C (desc.). HRMS (EI): 910,4388 (calculated for M^+ : 910, 4364). Elemental anal.: calculated (%) for $\text{C}_{50}\text{H}_{62}\text{N}_4\text{O}_{12}$: C 65,92; H 6,86; N 6,15;finding: C 65,99; H 6,71; N 6,00. HPLC: t = 8,6 min (ϕ = 1 ml/min; phosphates buffer: acetonitrile 1:1 a pH = 6.8). TLC: Rf: 0,39 (toluene:methanol:ethyl acetate 6:1:1). ^1H -RMN: 8,98 (NH-2); 8,18 (NH-3).

Example 3: Preparation of 4-deoxo-3,4-[(2R)-2-spiro(2-hydroxymethyl-3-methyl-6-p-methoxyphenyl-4-piperidil)-2,5-dihydro-1H-imidazolrifamycin S (1 RFA-2M)].

[0037] Following the general procedure of 1 RFA (1-2) from the compound 2b shown in Table 2 and the compound (3) shown in Schema 1, the compound of the title was obtained that presents the following data: P.f.: 171-74 °C (desc.). HRMS (ESI pos): 941.4546 [calculated for (M+1)⁺: 941. 4543]. Elemental anal.: calculated (%) for C₅₁H₆₄N₄O₁₃: C 65.09; H 6.85; N 5.95; found: C 65.22; H 6.79; N 5.82. HPLC: t = 11.9 min (Ø = 1 ml/min; phosphates buffer: acetonitrile 1:1 a pH = 6.8). TLC: Rf: 0.33 (toluene:methanol:ethyl acetate 6:1:1). ¹H-RMN: 8.99 (NH-2); 8.72 (NH-3).

Example 4: Preparation of 4-deoxo-3,4-[(2S)-2-spiro(2-hydroxymethyl-3-methyl-6-p-methoxyphenyl-4-piperidil)-2,5-dihydro-1H-imidazolrifamycin S (1 RFA-2m)].

[0038] Following the general procedure of 1 RFA (1-2) from the compound 2b shown in Table 2 and the compound (3) shown in Schema 1, the compound of the title was obtained that presents the following data: P.f.: 168-72 °C (desc.). HRMS (FAB⁺): 941.4543 (calculated for [M+1]⁺: 941. 4548). Elemental anal.: calculated (%) for C₅₁ H₆₄ N₄ O₁₃: C 65.09; H 6.85; N 5.95; found: C 65.30; H 6.74; N 5.78. HPLC: t = 9.1 min (Ø = 1 ml/min; phosphates buffer: acetonitrile 1:1 a pH = 6.8). TLC:Rf: 0.35 (toluene:methanol:ethyl acetate 6:1:1).¹H-RMN: 8.76 (NH-2); 9.47 (NH-3).

General method for the derivatives 1RFA (3-16) preparation:

[0039] It is dissolved 100 mg of 4-piperidone (2c-p) are dissolved in 15 ml of THF. It is added 1 equivalent of the intermediate 3-amino-4-iminorifamycin S (3)) is added, followed by 2 equivalents of ammonium acetate. The reaction is heated to reflux and it is monitored by TLC and HPLC until no detection of the starting materials (1-6 hours). The mixture is allowed to reach the room temperature and 30 ml of ether are added, leaving the dissolution to stir for 15 minutes. The mixture of reaction is extracted, by washing the organic layer three times with a buffer solution of (NH₄) H₂PO₄ (pH 7.6). The organic layer is dried with Na₂SO₄ anhydrous, filtered, and the solvents are removed at reduced pressure obtaining a solid of pink-violet colour formed mainly by a mixture of the two diastereoisomers looked for, whose proportion is obtained by HPLC (mixtures of acetonitrile-water) in reverse phase. The purification of the two diastereoisomers is carried out by preparative TLC using as eluents mixtures of: toluene:ethyl acetate:methanol / in a ratio of 6:1:1. The two diastereoisomers (violet compounds) separated in the plate are released from the support of glass with the help of a spatula and dissolved in chloroform. The silica gel is filtered and the solvents removed at reduced pressure, obtaining the two diastereoisomers (M/m) as violet solids. In the case that the diastereoisomers do not separate well, it is necessary to do a second purification by preparative TLC using eluent mixtures of hexane: THF:triethylamine / in a ratio of 5:4:1. The product is isolated by freeze drying, extraction and evaporation or crystallization of the selected chromatographic fractions.

[0040] Following this general method the compounds described in the Examples 5 to 32 were prepared.

Example 5: Preparation of 4-deoxo-3,4-[(2R)-2-spiro(2,6-diphenyl-4-piperidyl)-2,5-dihydro-1H-imidazo]rifamycin S (1 RFA-3M).

[0041] Following the general procedure of 1 RFA (1-2) from the compound 2c shown in Table 2 and the compound (3) shown in Schema 1, the compound of the title was obtained that presents the following data: P.f.:164-66 °C (desc.). HRMS (ESI pos):943.4469 [calculated for (M+1)⁺: 943. 4488]. Elemental anal.: calculated (%) for C₅₄H₆₂N₄O₁₁: C 68.77; H 6.63; N 5.94; found: C 68.47; H 6.59; N 6.22. HPLC: t = 11.2 min,(Ø = 1 ml/min ; Acetonitrile:water/65:35). TLC: Rf: 0.52 (toluene:methanol:ethyl acetate 6:1:1); 0.33 (hexane:THF:triethylamine 5:4:1) ¹H-RMN: 8.53 (NH-2); 8.99 (NH-3).

Example 6: Preparation of 4-deoxo-3,4-[(2S)-2-spiro(2,6-diphenyl-4-piperidyl)-2,5-dihydro-1H-imidazolrifamycin S (1 RFA-3m)].

[0042] Following the general procedure of 1 RFA (3-16) from the compound 2c shown in Table 2 and the compound (3) shown in Schema 1, the compound of the title was obtained that presents the following data: P.f.:168-70 °C (desc.). HRMS (ESI pos): 943.4469 [calculated for (M+1)⁺: 943.4488]. Elemental anal.: calculated (%) for C₅₄ H₆₂ N₄ O₁₁: C 68.77; H 6.63; N 5.94; found: C 68.63; H 6.69; N 5.99. HPLC: t = 9.6 min,(Ø = 1 ml/min ; Acetonitrile:water/65:35). TLC: Rf: 0.56 (toluene:methanol:ethyl acetate 6:1:1); 0.23 (hexane:THF:triethylamine 5:4:1). ¹H-RMN: 8.73 (NH-2); 9.98 (NH-3).

Example 7: Preparation of 4-deoxo-3,4-[(2R)-2-spiro[2,6-bis(4-methoxyphenyl)-4-piperidyl]-2,5-dihydro-1H-imidazolri-famycin S (1 RFA-4M).

[0043] Following the general procedure of 1 RFA (3-16) from the compound 2d shown in Table 2 and the compound (3) shown in Schema 1, the compound of the title was obtained that presents the following data: P.f.: 166-68 °C (desc.). HRMS (ESI pos): 1003.4706 [calculated for (M+1)+: 1003.4699]. Elemental anal.: calculated (%) for C₅₆H₆₆N₄O₁₃: C 67.05; H 6.63; N 5.59; found: C 66.87; H 6.43; N 5.48. HPLC: t = 8.2 min; (Ø = 1 ml/min; Acetonitrile:water/65:35). TLC: Rf: 0.49 (toluene:methanol:ethyl acetate 6:1:1); 0.25 (hexane:THF:triethylamine 5:4:1). ¹H-RMN: 8.56 (NH-2); 9.05 (NH-3).

Example 8: Preparation of 4-deoxo-3,4-[(2S)-2-spiro[2,6-bis(4-methoxyphenyl)-4-piperidil]-2,5-dihydro-1H-imidazolri-famycin S (1 RFA-4m).

[0044] Following the general procedure of 1 RFA (3-16) from the compound 2d shown in Table 2 and the compound (3) shown in Schema 1, the compound of the title was obtained that presents the following data: P.f.: 173-75 °C (desc.). HRMS (ESI pos): 1003.4691 [calculated for (M+1)+: 1003.4699]. Elemental anal.: calculated (%) for C₅₆H₆₆N₄O₁₃: C 67.05; H 6.63; N 5.59; found: C 66.92; H 6.71; N 5.20. HPLC: t = 7.2 min; (Ø = 1 ml/min; Acetonitrile:water/65:35). TLC: Rf: 0.54 (toluene:methanol:ethyl acetate 6:1:1); 0.18 (hexane:THF:triethylamine 5:4:1). ¹H-RMN: 8.65 (NH-2); 10.12 (NH-3).

Example 9: Preparation of 4-deoxo-3,4-[(2R)-2-spiro[2,6-bis(4-fluorophenyl)-4-piperidil]-2,5-dihydro-1H-imidazo]ri-famycin S (1 RFA-5M).

[0045] Following the general procedure of 1 RFA (3-16) from the compound 2e shown in Table 2 and the compound (3) shown in Schema 1, the compound of the title was obtained that presents the following data: P.f.: 172-75 °C (desc.). HRMS (ESI pos): 979.4292 [calculated for (M+1)+: 979.4299]. Elemental anal.: calculated (%) for C₅₄H₆₀F₂N₄O₁₁: C 66.24; H 6.18; N 5.72; found: C 66.15; H 6.16; N 5.47. HPLC: t = 10 min; (Ø = 1 ml/min; Acetonitrile:water/65:35). TLC: Rf: 0.55 (toluene:methanol:ethyl acetate 6:1:1); 0.30 (hexane:THF:triethylamine 5:4:1). ¹H-RMN: 8.70 (NH-2); 8.96 (NH-3).

Example 10: Preparation of 4-deoxo-3,4-[(2S)-2-spiro[2,6-bis(4-fluorophenyl)-4-piperidil]-2,5-dihydro-1 H-imidazo]ri-famycin S (1 RFA-5m).

[0046] Following the general procedure of 1 RFA (3-16) from the compound 2e shown in Table 2 and the compound (3) shown in Schema 1, the compound of the title was obtained that presents the following data: P.f.: 145 °C (desc.). HRMS (ESI pos): 979.4274 [calculated for (M+1)+: 979.4299]. Elemental anal.: calculated (%) for C₅₄H₆₀F₂N₄O₁₁: C 66.24; H 6.18; N 5.72; found: C 66.43; H 6.53; N 5.33. HPLC: t = 10.9 min; (Ø = 1 ml/min; Acetonitrile:water/65:35). TLC: Rf: 0.59 (toluene:methanol:ethyl acetate 6:1:1); 0.19 (hexane:THF:triethylamine 5:4:1). ¹H-RMN: 8.83 (NH-2); 9.94 (NH-3).

Example 11: Preparation of 4-deoxo-3,4-[(2R)-2-spiro[2,6-bis(2-iodophenyl)-4-piperidil]-2,5-dihydro-1 H-imidazo]ri-famycin S (1 RFA-6M).

[0047] Following the general procedure of 1 RFA (3-16) from the compound 2f shown in Table 2 and the compound (3) shown in Schema 1, the compound of the title was obtained that presents the following data: P.f.: 137-39 °C (desc.). HRMS (ESI pos): 1195.2430 [calculated for (M+1)+: 1195.2421]. Elemental anal.: calculated (%) for C₅₄H₆₀I₂N₄O₁₁: C 54.28; H 5.06; N 4.69; found: C 54.01; H 5.12; N 4.76. HPLC: t = 20.1 min; (Ø = 1 ml/min; Acetonitrile:water/65:35). TLC: Rf: 0.55 (toluene:methanol:ethyl acetate 6:1:1); 0.30 (hexane:THF:triethylamine 5:4:1). ¹H-RMN: 9.01 (NH-2); 8.63 (NH-3).

Example 12: Preparation of 4-deoxo-3,4-[(2S)-2-spiro[2,6-bis(2-iodophenyl)-4-piperidil]-2,5-dihydro-1H-imidazo]ri-famycin S (1 RFA-6m).

[0048] Following the general procedure of 1 RFA (3-16) from the compound 2f shown in Table 2 and the compound (3) shown in Schema 1, the compound of the title was obtained that presents the following data: HPLC: t = 28.7 min; (Ø = 1 ml/min; cetonitrile:water/65:35).

Example 13: Preparation of 4-deoxo-3,4-[(2R)-2-spiro(N-allyl-2,6-diphenyl-4-piperidil)-2,5-dihydro-1H-imidazo]rifamycin S (1 RFA-7M).

[0049] Following the general procedure of 1 RFA (3-16) from the compound 2g shown in Table 2 and the compound (3) shown in Schema 1, the compound of the title was obtained that presents the following data: P.f.: 175-77 °C (desc.). HRMS (ESI pos): 983.4778 [calculated for (M+1)⁺: 983.4801]. Elemental anal.: calculated (%) for C₅₇H₆₆N₄O₁₁: C 69.63; H 6.77; N 5.70; found: C 69.54; H 6.89; N 5.93. HPLC: t = 42.7 min; (Ø = 1 ml/min; acetonitrile:water/65:35). TLC: Rf: 0.55 (toluene:methanol:ethyl acetate 6:1:1); 0.32 (hexane:THF:triethylamine 5:4:1). ¹H-RMN: 8.62 (NH-2); 8.89 (NH-3).

Example 14: Preparation of 4-deoxo-3,4-[(2S)-2-spiro(N-allyl-2,6-diphenyl-4-piperidil)-2,5-dihydro-1H-imidazol-rifamycin S (1 RFA-7m).

[0050] Following the general procedure of 1 RFA (3-16) from the compound 2g shown in Table 2 and the compound (3) shown in Schema 1, the compound of the title was obtained that presents the following data: P.f.: 169-72 °C (desc.). HRMS (ESI pos): 983.4782 [calculated for (M+1)⁺: 983.4801]. Elemental anal.: calculated (%) for C₅₇H₆₆N₄O₁₁: C 69.63; H 6.77; N 5.70; found: C 69.32; H 7.09; N 6.08. HPLC: t = 45 min; (Ø = 1 ml/min; acetonitrile:water/65:35). TLC: Rf: 0.54 (toluene:methanol:ethyl acetate 6:1:1); 0.21 (hexane:THF: triethylamine 5:4:1). ¹H-RMN: 8.61 (NH-2); 10.28 (NH-3).

Example 15: Preparation of 4-deoxo-3,4-[(2R)-2-spiro(N-allyl-2,6-bis(4-methoxyphenyl)-4-piperidil)-2,5-dihydro-1H-imidazolrifamycin S (1 RFA-8M).

[0051] Following the general procedure of 1 RFA (3-16) from the compound 2h shown in Table 2 and the compound (3) shown in Schema 1, the compound of the title was obtained that presents the following data: P.f.: 158-61 °C (desc.). HRMS (ESI pos): 1043.5012 [calculated for (M+1)⁺: 1043.5012].

[0052] Elemental anal.: calculated (%) for C₅₉H₇₀N₄O₁₃: C 67.93; H 6.76; N 5.37; found: C 67.75; H 6.58; N 5.34. HPLC: t = 9.3 min; (Ø = 1 ml/min; acetonitrile:water/85:15). TLC: Rf: 0.46 (toluene:methanol:ethyl acetate 6:1:1); 0.29 (hexane:THF:triethylamine 5:4:1). ¹H-RMN: 8.65 (NH-2).

Example 16: Preparation of 4-deoxo-3,4-[(2S)-2-spiro(N-allyl-2,6-bis(4-methoxyphenyl)-4-piperidil)-2,5-dihydro-1H-imidazolrifamycin S (1 RFA-8m).

[0053] Following the general procedure of 1 RFA (3-16) from the compound 2h shown in Table 2 and the compound (3) shown in Schema 1, the compound of the title was obtained that presents the following data: P.f.: 157-59 °C (desc.). HRMS (ESI pos): 1043.5016 (calculated for [M+1]⁺: 1043.5012). Elemental anal.: calculated (%) for C₅₉H₇₀N₄O₁₃: C 67.93; H 6.76; N 5.37; found: C 68.13; H 6.83; N 5.12. HPLC t = 8.0 min; (Ø = 1 ml/min; acetonitrile:water/85:15). TLC: Rf: 0.49 (toluene:methanol:ethyl acetate 6:1:1); 0.18 (hexane:THF:triethylamine 5:4:1). ¹H-RMN: 8.74 (NH-2); 10.22 (NH-3).

Example 17: Preparation of 4-deoxo-3,4-[(2R)-2-spiro(N-allyl-2,6-bis(3,4-dimethoxyphenyl)-4-piperidil)-2,5-dihydro-1H-imidazo]rifamycin S (1 RFA-9M).

[0054] Following the general procedure of 1 RFA (3-16) from the compound 2i shown in Table 2 and the compound (3) shown in Schema 1, the compound of the title was obtained that presents the following data: HRMS (ESI pos): 1103.5229 [calculated for (M+1)⁺: 1103.5223]. Elemental anal.: calculated (%) for C₆₁H₇₄N₄O₁₅: C 66.41; H 6.76; N 5.08; found: C 66.18; H 6.80; N 5.17. HPLC: t = 4.9 min; (Ø = 1 ml/min; acetonitrile:water/85:15). TLC: Rf: 0.38 (toluene:methanol:ethyl acetate 6:1:1); 0.15 (hexane:THF:triethylamine 5:4:1). ¹H-RMN: 8.45 (NH-2); 8.95 (NH-3).

Example 18: Preparation of 4-deoxo-3,4-[(2S)-2-spiro(N-allyl-2,6-bis(3,4-dimethoxyphenyl)-4-piperidil)-2,5-dihydro-1H-imidazo]rifamycin S (1 RFA-9m).

[0055] Following the general procedure of 1 RFA (3-16) from the compound 2i shown in Table 2 and the compound (3) shown in Schema 1, the compound of the title was obtained that presents the following data: HRMS (ESI pos): 1103.5219 [calculated for (M+1)⁺: 1103.5223]. Elemental anal.: calculated (%) for C₆₁H₇₄N₄O₁₅: C 66.41; H 6.76; N 5.08; found: C 66.25; H 6.91; N 5.10. HPLC: t = 3.2 min; (Ø = 1 ml/min; acetonitrile:water/85:15). TLC: Rf: 0.43 (toluene:methanol:ethyl acetate 6:1:1); 0.08 (hexane:THF:triethylamine 5:4:1). ¹H-RMN: 8.59 (NH-2); 10.46 (NH-3).

Example 19: Preparation of 4-deoxo-3,4-[(2R)-2-spiro(N-allyl-2,6-bis(4-fluorophenyl)-4-piperidil)-2,5-dihydro-1H-imidazolrifamycin S (1 RFA-10M).

[0056] Following the general procedure of 1 RFA (3-16) from the compound 2j shown in Table 2 and the compound (3) shown in Schema 1, the compound of the title was obtained that presents the following data: P.f.: 139-41 °C (desc.). HRMS (ESI pos): 1019.4606 [calculated for (M+1)+: 1019.4612]. Elemental anal.: calculated (%) for C₅₇H₆₄F₂N₄O₁₁: C 67.18; H 6.33; N 5.50; found: C 67.00; H 6.44; N 5.71. HPLC: t = 5.7 min; (Ø = 1 ml/min; acetonitrile:water/85:15). TLC: Rf: 0.52 (toluene:methanol:ethyl acetate 6:1:1); 0.30 (hexane:THF:triethylamine 5:4:1). ¹H-RMN: 8.71 (NH-2); 8.82 (NH-3).

Example 20: Preparation of 4-deoxo-3,4-[(2S)-2-spiro(N-allyl-2,6-bis(4-fluorophenyl)-4-piperidil)-2,5-dihydro-1H-imidazolrifamycin S (1 RFA-10m).

[0057] Following the general procedure of 1 RFA (3-16) from the compound 2j shown in Table 2 and the compound (3) shown in Schema 1, the compound of the title was obtained that presents the following data: P.f.: 132-35 °C (desc.). HRMS (ESI pos): 1019.4591 [calculated for (M+1)+10.15 (NH-3). HPLC: t = 4.9 min; (Ø = 1 ml/min; acetonitrile:water/85:15). TLC: Rf: 0.52 (toluene:methanol:ethyl acetate 6:1:1); 0.15 (hexane:THF:triethylamine 5:4:1).

Example 21: Preparation of 4-deoxo-3,4-[(2R)-2-spiro(N-allyl-2,6-bis(4-chlorophenyl)-4-piperidil)-2,5-dihydro-1H-imidazolrifamycine S (1 RFA-11 M).

[0058] Following the general procedure of 1 RFA (3-16) from the compound 2k shown in Table 2 and the compound (3) shown in Schema 1, the compound of the title was obtained that presents the following data: P.f.: 165-68 °C (desc.). HRMS (ESI pos): 1051.4037 [calculated for (M+1)+: 1051.4021].

[0059] Elemental anal.: calculated (%) for C₅₇H₆₄Cl₂N₄O₁₁: C 65.07; H 6.13; N 5.33; found: C 65.37; H 6.04; N 5.28. HPLC: t = 8.6 min, (Ø=1; acetonitrile:water/85:15). TLC: Rf: 0.52 (toluene:methanol:ethyl acetate 6:1:1); 0.33 (hexane:THF:triethylamine 5:4:1). ¹H-RMN: 8.55 (NH-2); 8.84 (NH-3).

Example 22: Preparation of 4-deoxo-3,4-[(2S)-2-spiro(N-allyl-2,6-bis(4-chlorophenyl)-4-piperidil)-2,5-dihydro-1H-imidazolrifamycin S (1 RFA-11m).

[0060] Following the general procedure of 1 RFA (3-16) from the compound 2k shown in Table 2 and the compound (3) shown in Schema 1, the compound of the title was obtained that presents the following data: P.f.: 170-72 °C (desc.). HRMS (ESI pos): 1051.4021 [calculated for (M+1)+: 1051.4021]. Elemental anal.: calculated (%) for C₅₇H₆₄Cl₂N₄O₁₁: C 65.07; H 6.13; N 5.33; found: C 65.24; H 6.14; N 5.47. HPLC: t = 7.4 min; (Ø = 1 ml/min; acetonitrile:water/85:15). TLC: Rf: 0.54 (toluene:methanol:ethyl acetate 6:1:1); 0.13 (hexane:THF:triethylamine 5:4:1). ¹H-RMN: 8.67 (NH-2); 10.21 (NH-3).

Example 23: Preparation of 4-deoxo-3,4-[(2R)-2-spiro(N-allyl-2,6-bis(2-bromophenyl)-4-piperidil)-2,5-dihydro-1 H-imidazolrifamycin S (1 RFA-12M).

[0061] Following the general procedure of 1 RFA (3-16) from the compound 2l shown in Table 2 and the compound (3) shown in Schema 1, the compound of the title was obtained that presents the following data: P.f.: 187-89 °C (desc.). HRMS (ESI pos): 1139.3022 (calculated for (M+1)+: 1139.3011). Elemental anal.: calculated (%) for C₅₇H₆₄Br₂N₄O₁₁: C 60.00; H 5.65; N 4.91; found: C 60.34; H 5.33; N 4.80. HPLC t = 7.8 min; (Ø = 1 ml/min; acetonitrile:water/85:15). TLC: Rf: 0.52 (toluene:methanol:ethyl acetate 6:1:1); 0.26 (hexane:THF:triethylamine 5:4:1). ¹H-RMN: 8.74 (NH-2); 9.99 (NH-3).

Example 24: Preparation of 4-deoxo-3,4-[(2S)-2-spiro(N-allyl-2,6-bis(2-bromophenyl)-4-piperidil)-2,5-dihydro-1 H-imidazolrifamycin S (1 RFA-12m).

[0062] Following the general procedure of 1 RFA (3-16) from the compound 2l shown in Table 2 and the compound (3) shown in Schema 1, the compound of the title was obtained that presents the following data: P.f.: 182-85 °C (desc.).

[0063] HRMS (ESI pos): 1139.2980 [calculated for (M+1)+: 1139.3011]. Elemental anal.: calculated (%) for C₅₇H₆₄Br₂N₄O₁₁: C 60.00; H 5.65; N 4.91; found: C 60.24; H 5.65; N 4.79. HPLC: t = 8.9 min; (Ø = 1 ml/min; acetonitrile:water/85:15). TLC: Rf: 0.60 (toluene:methanol:ethyl acetate 6:1:1); 0.29 (hexane:THF:triethylamine 5:4:1). ¹H-RMN: 8.73 (NH-2); 10.08 (NH-3).

Example 25: Preparation of 4-deoxo-3,4-[(2R)-2-spiro(N-allyl-2,6-bis(3-bromophenyl)-4-piperidil)-2,5-dihydro-1H-imidazol-rifamycin S (1 RFA-13M).

[0064] Following the general procedure of 1 RFA (3-16) from the compound 2m shown in Table 2 and the compound (3) shown in Schema 1, the compound of the title was obtained that presents the following data: P.f.: 132-35 °C (desc.). HRMS (ESI pos): 1139.3015 [calculated for (M+1)+: 1139.3011]. Elemental anal.: calculated (%) for C₅₇H₆₄Br₂N₄O₁₁: C 60.00; H 5.65; N 4.91; found: C 60.15; H 5.58; N 4.93. HPLC: t = 11.7 min; (Ø = 1 ml/min; acetonitrile:water/85:15). TLC: Rf: 0.56 (toluene:methanol:ethyl acetate 6:1:1); 0.32 (hexane:THF:triethylamine 5:4:1). ¹H-RMN: 8.57 (NH-2); 8.74 (NH-3).

Example 26: Preparation of 4-deoxo-3,4-[(2S)-2-spiro(N-allyl-2,6-bis(3-bromophenyl)-4-piperidil)-2,5-dihydro-1 H-imidazo]-rifamycin S (1 RFA-13m).

[0065] Following the general procedure of 1 RFA (3-16) from the compound 2m shown in Table 2 and of the compound (3) shown in Schema 1, the compound of the title was obtained that presents the following data: P.f.: 140-42 °C (desc.). HRMS (ESI pos): 1139.3012 [calculated for (M+1)+: 1139.3011]. Elemental anal.:calculated (%) for C₅₇H₆₄Br₂N₄O₁₁: C 60.00; H 5.65; N 4.91; found: C 60.05; H 5.79; N 4.68. ¹H-RMN: 8.59 (NH-2); 9.01 (NH-3). HPLC t = 9.7 min, (Ø = 1 ml/min; acetonitrile:water/85:15). TLC: Rf: 0.57 (toluene:methanol:ethyl acetate 6:1:1); 0.11 (hexane:THF:triethylamine 5:4:1). ¹H-RMN: 8.74 (NH-2); 9.99 (NH-3).

Example 27: Preparation of 4-deoxo-3,4-[(2R)-2-spiro(N-allyl-2,6-bis(2-iodophenyl)-4-piperidil)-2,5-dihydro-1 H-imidazo]-rifamycin S (1 RFA-14M).

[0066] Following the general procedure of 1 RFA (3-16) from the compound 2n shown in Table 2 and the compound (3) shown in Schema 1, the compound of the title was obtained that presents the following data: P.f.: 163-64 °C (desc.). HRMS (ESI pos):1235.2716 [calculated for (M+1)+: 1235.2734]. Elemental anal.:calculated (%) for C₅₇H₆₄I₂N₄O₁₁: C 55.44; H 5.22; N 4.54; found: C 55.05; H 5.19; N 4.15. HPLC: t = 10.0 min; (Ø = 1 ml/min; acetonitrile:water/85:15). TLC: Rf: 0.53 (toluene:methanol:ethyl acetate 6:1:1); 0.28 (hexane:THF:triethylamine 5:4:1). ¹H-RMN: 8.74 (NH-2); 8.91 (NH-3).

Example 28: Preparation of 4-deoxo-3,4-[(2S)-2-spiro(N-allyl-2,6-bis(2-iodophenyl)-4-piperidil)-2,5-dihydro-1H-imidazo]rifamycin S (1 RFA-14m).

[0067] Following the general procedure of 1 RFA (3-16) from the compound 2n shown in Table 2 and the compound (3) shown in Schema 1, the compound of the title was obtained that presents the following data: P.f.:149-51 oC (desc.). UV (λ = 254 nm): 214.5; 280; 320. HRMS (ESI pos): 1235.2701 [calculated for (M+1)+: 1235.2734]. Elemental anal.: calculated (%) for C₅₇H₆₄I₂N₄O₁₁: C 55.44; H 5.22; N 4.54; found: C 55.06; H 5.03; N 4.16. HPLC t = 13.0 min; (Ø = 1 ml/min; acetonitrile:water/65:35). TLC: Rf: 0.60 (toluene:methanol:ethyl acetate 6:1:1); 0.32 (hexane:THF:triethylamine 5:4:1). ¹H-RMN: 8.82 (NH-2); 10.34 (NH-3).

Example 29: Preparation of 4-deoxo-3,4-[(2R)-2-spiro(N-homoallyl-2,6-diphenyl-4-piperidil)-2,5-dihydro-1H-imidazol-rifamycin S (1 RFA-15M).

[0068] Following the general procedure of 1 RFA (3-16) from the compound 2o shown in Table 2 and the compound (3) shown in Schema 1, the compound of the title was obtained that presents the following data: HRMS (ESI pos): 997.4953 [calculated for (M+1)+: 997.4957]. Elemental anal.: calculated (%) for C₅₈H₆₈N₄O₁₁: C 69.86; H 6.87; N 5.62; found: C 69.73; H 6.92; N 5.71. HPLC t = 29.3 min, (Ø = 1 ml/min; acetonitrile:water/65:35). TLC: Rf: 0.56 (toluene:methanol:ethyl acetate 6:1:1); 0.36 (hexane:THF:triethylamine 5:4:1). ¹H-RMN: 8.66 (NH-2); 8.83 (NH-3).

Example 30: Preparation of 4-deoxo-3,4-[(2S)-2-spiro(N-homoallyl-2,6-diphenyl-4-piperidil)-2,5-dihydro-1 H-imidazo]rifamycin S (1 RFA-15m).

[0069] Following the general procedure of 1 RFA (3-16) from the compound 2o shown in Table 2 and the compound (3) shown in Schema 1, the compound of the title was obtained that presents the following data: HRMS (ESI pos): 997.4951 [calculated for (M+1)+: 997,4957]. Elemental anal.: calculated (%) for C₅₈H₆₈N₄O₁₁: C 69.86; H 6.87; N 5.62; found: C 69.99; H 6.89; N 5.43. HPLC t = 27.0 min; (Ø = 1 ml/min; acetonitrile:water/65:35). TLC: Rf: 0.59 (toluene:methanol:ethyl acetate 6:1:1); 0.20 (hexane:THF:triethylamine 5:4:1). ¹H-RMN: 8.70 (NH-2)10.26 (NH-3).

Example 31: Preparation of 4-deoxo-3,4-[(2R)-2-spiro(N-buthyl-2,6-diphenyl-4-piperidil)-2,5-dihydro-1H-imidazolri-famycin S (1 RFA-16M).

[0070] Following the general procedure of 1 RFA (3-16) from the compound 2p shown in Table 2 and the compound (3) shown in Schema 1, the compound of the title was obtained that presents the following data: P.f.: 160-63 °C (desc.). UV ($\lambda = 254$ nm): 240, 278, 320. HRMS (ESI pos): 999.5087 [calculated for (M+1)+: 999.5114]. Elemental anal.: calculated (%) for $C_{58}H_{70}N_4O_{11}$: C 69.72; H 7.06; N 5.61; found: C 69.76; H 7.36; N 5.47. HPLC: t = 5.3 min, ($\phi = 1$ ml/min; acetonitrile:water/85:15). TLC: Rf: 0.54 (toluene:methanol:ethyl acetate 6:1:1); 0.38 (hexane:THF:triethylamine 5:4:1). 1H -RMN: 8.72 (NH-2) 8.90 (NH-3).

Example 32: Preparation of 4-deoxo-3,4-[(2S)-2-spiro(N-buthyl-2,6-diphenyl-4-piperidil)-2,5-dihydro-1 H-imidazolri-famycin S (1 RFA-16m).

[0071] Following the general procedure of 1 RFA (3-16) from the compound 2p shown in Table 2 and the compound (3) shown in Schema 1, the compound of the title was obtained that presents the following data: P.f.: 156-58 °C (desc.). UV ($\lambda = 254$ nm): 239, 278, 320. HRMS (ESI pos): 999.5102 [calculated for (M+1)+: 999.5114]. TLC: Rf: 0.60 (toluene: methanol:ethyl acetate 6:1:1); 0.20 (hexane:THF:triethylamine 5:4:1). 1H -RMN: 8.63 (NH-2) 10.5 (NH-3)

Example 33: Results of the biological activity studies

[0072] The studies were carried out using the method of the proportions (reference method) and following the protocol described by the NCCLS (National Committee for Clinical Laboratory Standards). The culture media was prepared using Middlebrook 7H10 supplemented with 10% OADC (oleic acid-albumin-dextroxe-catalase). For each compound 2 inoculums were tested (equivalent to the $n^{\circ}1$ of McFarland and a dilution of 1/100) trying to obtain an ideal inoculum that contains among 100-200 colonies. The plates were incubated in heater at 37°C, for 21 days under a 5% to 10% CO₂ atmosphere.

In the studies carried out, the behaviour of the compounds analysed demonstrated in vitro a strong antimycobacteri-alantimycobacterial general activity (minimum inhibitory concentration, MIC < 0,1 μ g/ml) and this activity was found to be more effective than rifampicin-rifabutin in the strain of reference Mycobacterium tuberculosis ATCC 37837 that accredits a high grade of resistance to rifampicin. It also emphasized the in vitro behaviour of the compounds in five (5) strains of Mycobacterium avium-intracellulare complex analysed, where five strains were sensitive to at least three (3) of the compounds of formula (1) studied. On the contrary, all of them manifested resistance to rifampicin-rifabutin (MIC > 1 μ g/ml). As for the 4 strains of Mycobacterium kansasii all the compounds of formula (1) studied had a similar behaviour to rifampicin-rifabutin.

[0073] Table 3 includes the results obtained in the study of biologic activity of the spiropiperidynilrifamycins of general formula (1). s means sensitive, R means resistant and s/R means sensitive to some strains and resistant to others, Each test has been assayed using the compound on several different Mycobacterium strains, for example, as in entry 11 of the table, five (5) strains have been used.

[0074] If the plate that contains the antibiotic exceeds a 1% of the number of colonies of the control plate (without antibiotic), the microorganism is considered R, in the case of being less than 1% is considered s.

[0075] Entries 1-5 show the results obtained in front of strains of collection (each one resists to one of the commercial drugs assayed: pyrazinamide PZ, isoniazid IZ, ethambutol EB, rifampicin RI and 2-thiophene hydrazo carboxylic acid TCH), this last drug is used as a control as most Mycobacterium are resistant to it. The entries 6-10 show the results obtained in 52 strains assayed, classified in function by resistance observed to commercial drugs. The entries 11 and 12 show the results obtained from 9 atypical Mycobacterium strains.

Example 34: Biological activity studies in strains of multiresistant Mycobacterium tuberculosis.

[0076] Using the same experimental method that the described in Example 33, assays have shown in vitro effectiveness of the compounds of formula (1) exclusively in strains of multiresistant Mycobacterium tuberculosis. These strains have been provided by the Microbiological Service of the "Gregorio Marañón" General University Hospital, Madrid; and the Supranational Reference Laboratory Network of the World Health Organization (Mycrobiological Service, Hospital University Vall d'Hebron, Barcelona). The compounds 1 RFA 3M and 1 RFA 5M, have been tested against multiresistant Mycobacterium tuberculosis because they have shown less MIC in the preliminary assays. The obtained results proved a high antimycobacterial activity of the two compounds assayed, much superior to rifampicin and to rifabutin. At a concentration > 0.5 μ g/ml they inhibit growth of more than 75% of resistant strains to IZ and and RI (MIC > 256 μ g/ml).

[0077] On the other hand, the rifabutin, at a concentration of 1 μ g/ml shows a superior sensitivity of 14.3% to rifampicin but much lower to 1 RFA 3M and 1 RFA 5M (95.2% and 100% respectively). Table 4 shows collected results

EP 1 783 129 A1

of said assays of biological activity of the compounds 1 RFA 3M and 1 RFA 5M. The entry 1 shows the results obtained in four (4) strains resistant to rifampicin. The entry 2 shows the results obtained in seventeen (17) multiresistant strains (Defined here by resistance to resistance to at least isoniazid and rifampicin).).

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Table 3. Assays of biological activity.

n°	Strain Concentration analysed µg/ml	PZ	IZ	EB	RI	SM	TC H	RFB	RFA 7M	RFA 4M	RFA 3M	RFA 3m	RFA 5M	RFA 15M	RFA 16M	Control
1	Mycobacterium tuberculosis ATCC 35837	s	s	s	R	R	R	R	R	s	s	s	s	R	s	Ok
2	Mycobacterium tuberculosis ATCC 35820	s	s	s	s	R	R	s	s	s	s	s	s	s	s	Ok
3	Mycobacterium tuberculosis ATCC 35838	s	s	R	s	s	R	s	s	s	s	s	s	s	s	Ok
4	Mycobacterium tuberculosis ATCC 35822	s	R	s	s	s	R	s	s	s	s	s	s	s	s	Ok
5	Mycobacterium tuberculosis H37 RV	s	s	s	s	s	R	s	s	s	s	s	s	s	s	Ok
6	Mycobacterium tuberculosis 38 strains assayed	s	s	s	s	s	R	s	s	s	s	s	s	s	s	Ok
7	Mycobacterium tuberculosis 8 strains assayed	R	s	s	s	s	R	s	s	s	s	s	s	s	s	Ok
8	Mycobacterium tuberculosis 2 strains assayed	s	R	s	s	s	R	s	s	s	s	s	s	s	s	Ok
9	Mycobacterium tuberculosis 3 strains assayed	s	s	s	s	R	R	s	s	s	s	s	s	s	s	Ok

(continued)

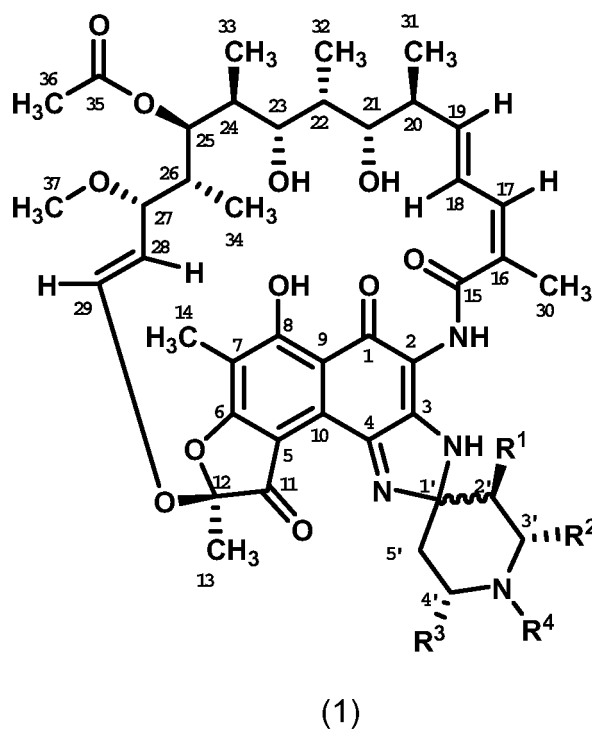
n°	Strain	PZ	IZ	EB	RI	SM	TC H	RF B	RFA 7M	RFA 4M	RFA 3M	RFA 3m	RFA 5M	RFA 15M	RFA 16M	Control
10	Concentration analysed µg/ml Mycobacterium tuberculosis 1 strain assayed	25	0.2	5	1	4	1	1	1	1	1	1	1	1	1	Ok
11	Mycobacterium avium-intracellulare complex 5 strains assayed	R	R	R	s	R	R	R	s	s/R	s	s	s	s/R	s/R	Ok
12	Mycobacterium Kansaii 4 strains assayed	R	R	R	s	R	R	s	s	s	s	s	s	s	s	Ok

Table 4: Assays of biological activity in strains of multiresistant *Mycobacterium tuberculosis*

				n° (% Sensitivity)					
		IZ	RI	1RFA 3M			1 RFA 5M		
	STRAIN (Concentration analyzed $\mu\text{g/ml}$)	(0,2)	(1)	(0,1)	(0,5)	(1)	(0,1)	(0,5)	(1)
1	<i>Mycobacterium tuberculosis</i> 4 strains assayed	S	R	1 (25)	2 (50)	4 (100)	1 (25)	2 (50)	4 (100)
2	<i>Mycobacterium tuberculosis</i> multiresistant 17 strains assayed	R	R	4 (23)	13 (75)	16 (94)	3 (17)	14 (82)	17 (100)

Claims

1. A compound of formula (1),



their pharmaceutically acceptable salts and their solvates, including hydrates, wherein:

R^1 is a radical selected from hydrogen and (C_1-C_4) -alkyl;

R^2 is selected from the group consisting of hydroxy- (C_1-C_4) -alkyl, phenyl, mono-substituted phenyl and phenyl di-substituted at the positions 3 and 4, the substituents being selected from the group consisting of halogen, (C_1-C_4) -alkyl and (C_1-C_4) -alkoxy;

R^3 is selected from the group consisting of phenyl, mono-substituted phenyl and phenyl di-substituted at the positions 3 and 4, the substituents being selected from the group consisting of halogen, (C_1-C_4) -alkyl and (C_1-C_4) -alkoxy; and

R^4 is selected from the group consisting of hydrogen, (C_1-C_4) -alkyl; allyl, and homoallyl.

2. The compound according to claim 1, wherein when R^2 and R^3 are mono- or di-substituted phenyl, the substituents

of the phenyl are independently selected between halogen and (C₁-C₄)-alkoxyl.

3. The compound according to claim 2, wherein the alkoxyl is a methoxyl.

4. The compound according to claim 1, wherein R¹ is methyl; R² is hydroxymethyl; R³ is phenyl; and R⁴ is hydrogen.

5. The compound according to claim 1, wherein R¹ is methyl; R² is hydroxymethyl; R³ is 4-methoxyphenyl; and R⁴ is hydrogen.

6. The compound according to claim 1, wherein R¹ and R⁴ are hydrogen; and R² and R³ are phenyl.

7. The compound according to claim 1, wherein R¹ and R⁴ are hydrogen; and R² and R³ are 4-methoxyphenyl.

8. The compound according to claim 1, wherein R¹ and R⁴ are hydrogen; and R² and R³ are 4-fluorophenyl.

9. The compound according to claim 1, wherein R¹ and R⁴ are hydrogen; and R² and R³ are 2-iodophenyl.

10. The compound according to claim 1, wherein R¹ is hydrogen; R² and R³ are phenyl; and R⁴ is allyl.

11. The compound according to claim 1, wherein R¹ is hydrogen; R² and R³ are 4-methoxyphenyl; and R⁴ is allyl.

12. The compound according to claim 1, wherein R¹ is hydrogen; R² and R³ are 3,4-dimethoxyphenyl; and R⁴ is allyl.

13. The compound according to claim 1, wherein R¹ is hydrogen; R² and R³ are 4-fluorophenyl; and R⁴ is allyl.

14. The compound according to claim 1, wherein R¹ is hydrogen; R² and R³ are 4-chlorophenyl; and R⁴ is allyl.

15. The compound according to claim 1, wherein R¹ is hydrogen; R² and R³ are 2-bromophenyl; and R⁴ is allyl.

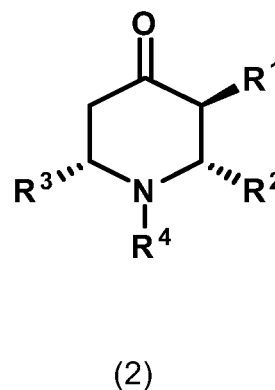
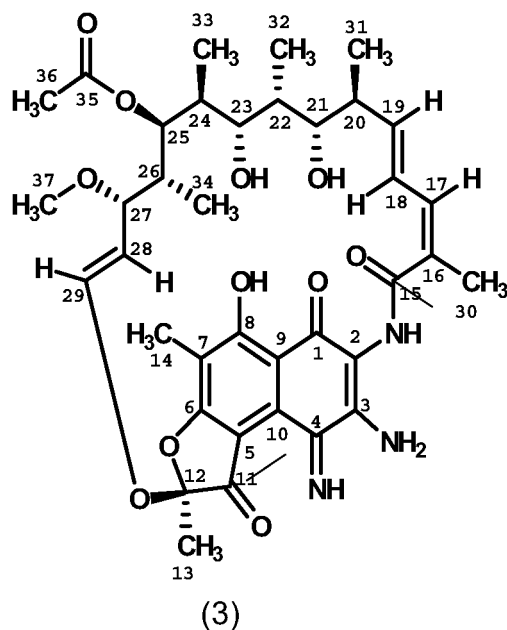
16. The compound according to claim 1, wherein R¹ is hydrogen; R² and R³ are 3-bromophenyl; and R⁴ is allyl.

17. The compound according to claim 1, wherein R¹ is hydrogen; R² and R³ are 2-iodophenyl; and R⁴ is allyl.

18. The compound according to claim 1, wherein R¹ is hydrogen; R² and R³ are phenyl; and R⁴ is homoallyl.

19. The compound according to claim 1, wherein R¹ is hydrogen; R² and R³ are phenyl; and R⁴ is buthyl.

20. A process for the preparation of the compound according to claim 1, that comprises reacting, in a suitable solvent, a 3-amino-4-iminorifamycin S of formula (3) with a substituted 4-piperidone of formula (2) wherein R¹, R², R³ and R⁴ have the same meaning that in claim 1.



21. The process according to claim 20, wherein the solvent is a cyclic ether.
22. The process according to claim 21, wherein the cyclic ether is tetrahydrofurane.
23. The process according to any of the claims 20-22, wherein the reaction is carried out in presence of a base.
24. The process according to claim 23, wherein the base is ammonium acetate.
25. The process according to any of the claims 20-23, wherein the temperature to which the reaction is carried out is the reflux temperature of the solvent.
26. Use of the compound defined in any of the claims 1-19 for the preparation of a medicament for the treatment of a mycobacterial infection.
27. Use according to claim 26, wherein the mycobacterial infection is produced by a bacterium selected from the group consisting of Mycobacterium tuberculosis, Mycobacterium avium-intracellulare complex, and Mycobacterium kansasii.
28. A method of treatment of a mammal that suffers a mycobacterial infection, including a human, that comprises the administration to said mammal of a therapeutically effective quantity of a compound defined in any of the claims 1-19, together with suitable quantities of pharmaceutically acceptable excipients or carriers.
29. The method according to claim 28, wherein the mycobacterial infection is produced by a bacterium selected from the group consisting of Mycobacterium tuberculosis, Mycobacterium avium-intracellulare complex, and Mycobacterium kansasii.
30. A Pharmaceutical composition for the treatment of mycobacterial infections, that comprises a therapeutically effective quantity of the compound defined in any of the claims 1-19, together with pharmaceutically acceptable excipients or carriers.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/ ES 2005/000380

A. CLASSIFICATION OF SUBJECT MATTER		
See additional sheet		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols)		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)		
CIBEPAT.EPODOC. REGISTRY. BEILSTEIN. HCAPLUS. WPI		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	RUBIO, E. y col. NMR spectroscopic analysis of new spiro-piperidylrifamycins. Magnetic Resonance in Chemistry. April 2005, Vol. 43, N°4, pages 269-282. The whole document	1,2,3,4,5,17, 20-30
A	US 4219478 A (MARSILI, L. y col.) 26.08.1980 The whole document, especially examples 4, 5, 7, 12-23	1-30
☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.		
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Date of the actual completion of the international search 23 September 2005 (23.09.05)		Date of mailing of the international search report 28 September 2005 (28.09.05)
Name and mailing address of the ISA/ S.P.T.O.		Authorized officer
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C 07D 491/20 (2006.01)
C07D 235:14 (2006.01)
C07D 307:83 (2006.01)

INTERNATIONAL SEARCH REPORT
 Information on patent family members

 International Application No
 PCT/ ES 2005/000380

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 4219478 A	26.08.1980	PT 65208 AB	01.07.1976
		BE 842883 A1	01.10.1976
		IE 42998 B1	03.12.1980
		SE 7606640 A	14.12.1976
		DK 258076 A	14.12.1976
		NO 762026 A	14.12.1976
		FI 761688 A	14.12.1976
		NL 7606370 A	15.12.1976
		DE 2626296 A1	30.12.1976
		FR 2314189 A1	07.01.1977
		JP 52036683 A	22.03.1977
		ZA 7603256 A	25.05.1977
		ES 449187 A1	16.07.1977
		AR 211127 A1	31.10.1977
		AU 1476976 A	15.12.1977
		AT 411276 A	15.12.1977
		IN 143526 A1	17.12.1977
		HU 171396 B	28.01.1978
		US 4086225 A	25.04.1978
		PL 99493 B	31.07.1978
		GB 1542063 A	14.03.1979
		CA 1059123 A1	24.07.1979
		SU 680649 A3	15.08.1979
		IL 49701 A	31.10.1979
		DE 2825445 A1	20.12.1979
		CS 197271 B1	30.04.1980
		CH 625524 A5	30.09.1981
		IT 1056272 B	30.01.1982
		YU 140576 A1	30.06.1982
		HK 56083 A	25.11.1983
		SI 7611405 A8	30.04.1997

Form PCT/ISA/210 (patent family annex) (July 1992)

REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

- US 4086225 A [0010]
- DE 2825445 A [0010] [0018]
- US 4219478 A [0010]
- US 4017481 A [0018]

Non-patent literature cited in the description

- **P. SENSI et al.** *Il Fármaco, Ed. Sc.*, 1959, vol. 14, 146-147 [0006]
- **C. BARTOLUCCI et al.** *Fármaco*, 1992, vol. 47, 1367 [0006]
- **C. BARTOLUCCI et al.** *Pharm. Pharmacol. Lett.*, 1993, vol. 3, 1 [0006]
- **P. SENSI.** *P. Appl. Chem.*, 1975, vol. 41, 15-29 [0008]
- **S. LANCINI et al.** *Structure-Activity Relationship among the Semisynthetic Antibiotics.* Academic Press, 1977, 531-600 [0009]
- **A. SANFILIPPO et al.** *J. Antib.*, 1980, vol. 33, 1193 [0010]
- **L. MARSILI et al.** *J. Antib.*, 1981, vol. 34, 1033 [0010]
- **J. BARLUENGA et al.** *J. Org. Chem.*, 1993, vol. 58, 3391 [0018]
- **J. BARLUENGA et al.** *J. Org. Chem.*, 1998, vol. 63, 3918 [0018]
- **A-B. GARCÍA et al.** *Tetrah. Letter*, 2004, vol. 45, 4357 [0018]
- **E. RUBIO et al.** *Magn. Reson. Chem.*, 2005, vol. 43, 269-282 [0024]